



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification<sup>6</sup> :

A61K 45/06

A1

(11) International Publication Number:

WO 97/31654

(43) International Publication Date:

4 September 1997 (04.09.97)

(21) International Application Number: PCT/EP97/00873

(22) International Filing Date: 24 February 1997 (24.02.97)

(30) Priority Data:

MI96A000352

26 February 1996 (26.02.96)

IT

(71) Applicant (for all designated States except US): NICOX S.A.  
[FR/FR]; 45, avenue Kléber, F-75116 Paris (FR).

(72) Inventor; and

(75) Inventor/Applicant (for US only): DEL SOLDATO, Piero  
[IT/IT]; Via Toti, 22, I-20052 Monza (IT).(74) Agents: SAMA, Daniele et al.; Sama Patents, Via G.B.  
Morgagni, 2, I-20129 Milano (IT).

(81) Designated States: AL, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: NITRIC OXIDE DONORS CAPABLE OF REDUCING TOXICITY FROM DRUGS

(57) Abstract

Use of organic compounds containing the -ONO<sub>2</sub> function, or inorganic compounds containing the -NO group or compositions comprising said compounds to reduce the toxicity caused by drugs to the gastrointestinal and/or renal apparatus, said compounds being characterized in that they are nitric oxide NO donors, i.e. when they are put into contact in vitro with cells of the basal endothelium or platelets.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## NITRIC OXIDE DONORS CAPABLE OF REDUCING TOXICITY FROM DRUGS

\* \* \* \*

The present invention relates to the prevention or the reduction of the iatrogenic toxicity. More particularly it relates to the reduction of toxicity caused by drugs at renal and/or gastrointestinal and/or respiratory level.

It is well known that the toxicity from drugs is assuming a more and more important role in human pathology. It suffices to consider the gastropathy caused by anti-inflammatory drugs which implies an yearly cost in the range of some billions of dollars for the U.S. public administration. See for instance Bloom, B.S. Am. J. Medicine 84 (supplement 2A), 20, 1988, which reports the yearly costs for the arthritis treatment in USA amounting to more than 12 billions of dollars, of which more than 30% is attributable to the care of the side effects connected to the anti-inflammatory/antiarthritic pharmacological treatment.

Likewise the nephropathy caused by antibiotics can mean for the single patient losses of thousands of dollars to cover hospitalization expenses. See for instance Berndt W.O. et al. in "Principles of Pharmacology" Munson P.L. Ed. p. 685, 1995.

An object of the present invention consists in compounds capable of reducing the toxicity caused by non nitro-

derivative drugs to the gastrointestinal and/or renal and/or respiratory apparatus.

It has been surprisingly and unexpectedly found that this is possible if organic compounds containing the  $-\text{ONO}_2$  function, or inorganic compounds containing the  $-\text{NO}$  group are employed, said compounds being characterized in that they are nitric oxide NO donors, i.e. when they are put into contact in vitro with cells of the vasal endothelium, platelets, etc., and after incubation of 5 minutes at the temperature of  $37^\circ\text{C}$  are capable of releasing NO and activating the cGMP (Guanosine cyclic 3',5'-(hydrogen phosphate)) synthesis, as determined by the specific tests utilized, which will be described in detail in the examples.

The unexpected and surprising results of the claimed invention are also shown by the following fact: the combination of the nitroderivatives of the invention with a non nitroderivative drug is useful not only to reduce the toxicity of the drug but also to eliminate the disadvantages related to the nitroderivatives administration.

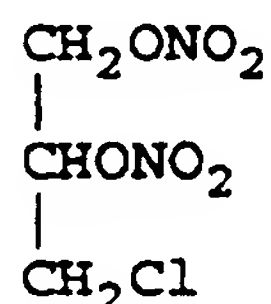
For example nitroglycerin, when given with enalapril to rats, following repeated subcutaneous administration at the dose of 1 mg/kg per day, did not cause any tolerance, differently from nitroglycerin alone.

Therefore the combination of the present invention results in the so called lower tolerance by chronical administration

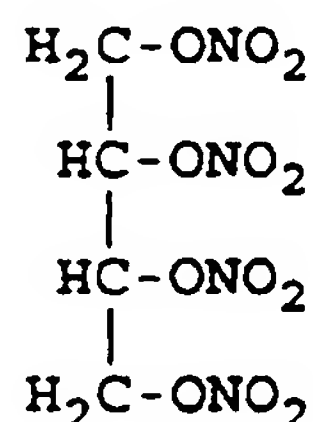
pharmaceutical compositions. This is a great advantage since no problem arises also by taking nitroderivatives for a long time and maintaining the same effectiveness of the nitroderivative compounds.

The organic compounds containing  $-\text{ONO}_2$  functions which can be mentioned as an example, are the following, which are reported in The Merck Index 11th Ed. - 1989 and prepared with the known methods, for instance those reported in the Merck, incorporated herein by reference.:

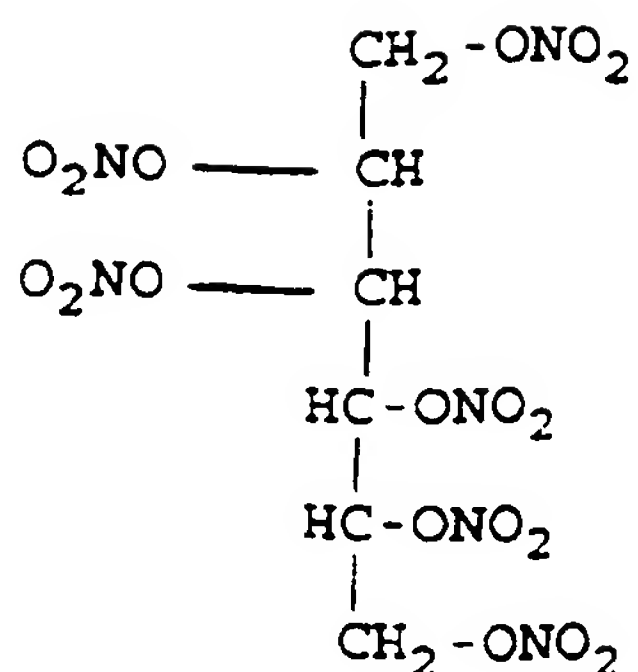
clonitrate (3-chloro-1,2-propanediol dinitrate) (Merck No. 2390) having the formula  $\text{C}_3\text{H}_5\text{ClN}_2\text{O}_6$  and formula of structure



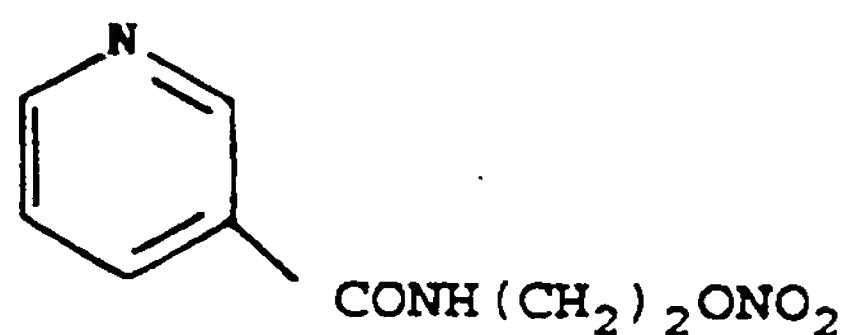
erythrityltetranitrate (1,2,3,4 butanetetroltetranitrate) (Merck No. 3622) having the formula  $\text{C}_4\text{H}_6\text{N}_4\text{O}_{12}$  and formula of structure



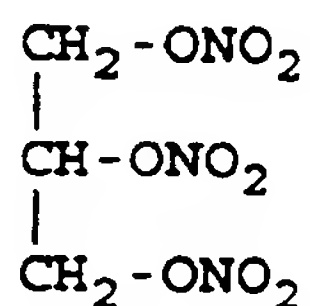
mannitol hexanitrate (Merck No. 5630) having the formula  $\text{C}_6\text{H}_8\text{N}_6\text{O}_{18}$  and formula of structure



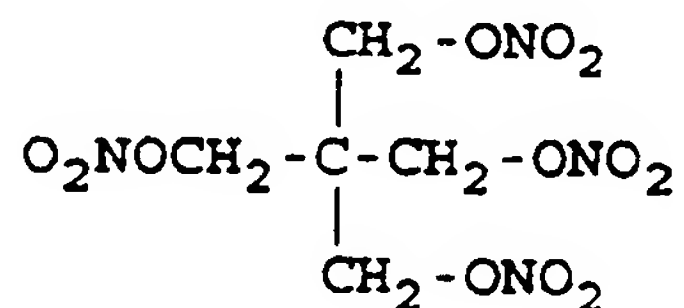
nicorandil (N-[2-(nitrooxy)ethyl]-3-pyridine-carboxamide)  
 (Merck No. 6431) having the formula  $\text{C}_8\text{H}_9\text{N}_3\text{O}_4$  and formula of  
 structure



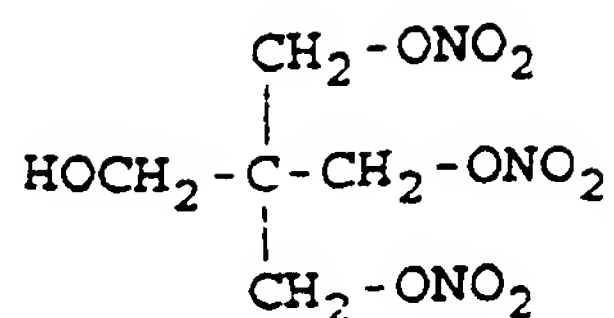
nitroglycerin (1,2,3 propanetriol trinitrate) (Merck No.  
 6528) having the formula  $\text{C}_3\text{H}_5\text{N}_3\text{O}_9$  and formula of structure



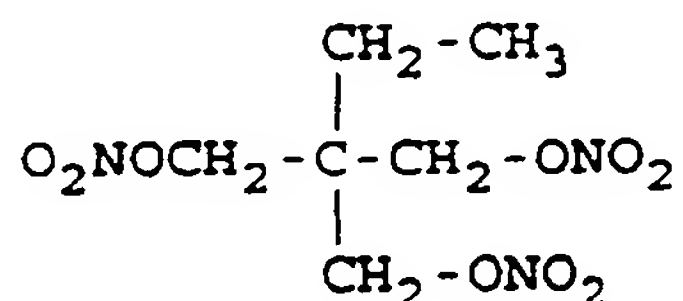
pentaerythritoltetranitrate (2,2-bis [(nitrooxy)-methyl]-  
 1,3-propanedioldinitrate) (Merck No. 7066) having the  
 formula  $\text{C}_5\text{H}_8\text{N}_4\text{O}_{12}$  and formula of structure



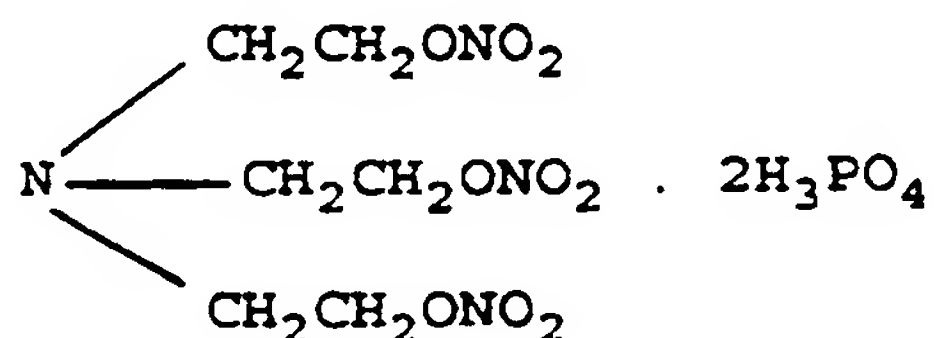
pentrinitrol (2,2-bis[(nitrooxy)methyl]-1,3-propanediolmono-  
 nitrate) (Merck No. 7094) having the formula  $\text{C}_5\text{H}_9\text{N}_3\text{O}_{10}$  and  
 formula of structure



propatylnitrate (2-ethyl-2-[(nitrooxy)methyl]-1,3-propanedioldinitrate) (Merck No. 7821) having the formula  $\text{C}_6\text{H}_{11}\text{N}_3\text{O}_9$  and formula of structure



trolnitratephosphate (2,2',2"-nitryltrisethanoltrinitrate phosphate) (salt 1:2) (Merck No. 9682) having the formula  $\text{C}_6\text{H}_{18}\text{N}_4\text{O}_{17}\text{P}_2$  and formula of structure



Among the inorganic compounds containing the -NO group, nitroprussiates can be mentioned, such as for instance:

sodiumnitroprussiate (pentakis (cyano-C)nitrosylferrate (2-)disodium) (Merck No. 8600) having the formula  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ .

Other compounds containing the  $\text{-ONO}_2$  function are reported in patent applications in the name both of the Applicant WO 95/30641; WO 95/09831; WO 94/12463 and of others WO 94/04484. These patent applications PCT/WO are herein incorporated by reference both for the compounds and for the preparation processes.

The nitric oxide NO donors compounds of the invention are indicated hereinafter by the term DON-NO.

Among the drugs not containing nitrodrivative groups causing renal and/or gastrointestinal and/or respiratory toxicity, the following compounds belonging to different therapeutic classes, can be mentioned, as an example:

anti-tumoral drugs among which cisplatin, 5 fluoro-uracil can be mentioned;

immunodepressive drugs among which cyclosporin can be cited;

anti-viral drugs among which acyclovir can be cited;

non-steroidal anti-inflammatory drugs, among which ibuprofen, indomethacin, diclofenac, ketorolac, naproxen, ketoprofen, mefenamic acid, flunixin, flufenamic acid, niflumic acid can be mentioned;

anti-thrombotic drugs among which aspirin can be mentioned;

steroidal anti-inflammatory drugs among which cortisone, dexamethasone, methylprednisolone can be mentioned;

antibiotics among which ciprofloxacin, gentamicine can be mentioned;

inhibitors of the angiotensin-converting enzyme (ACE) among which captopril, enalapril can be mentioned;

beta-adrenergic antagonists, e.g. atenolol, metoprolol, timolol, propanol, etc. Also for these agents respiratory toxicity was reduced by the administration of the nitroderivatives of the invention.



All these drugs are reported in the Merck Index (see above) herein incorporated by reference.

The preferred compounds as drugs not containing the nitroderivative group of which it is desired to prevent or reduce the toxicity, are antitumoral drugs, in particular cis-platinum (cisplatin); immunodepressive drugs, in particular cyclosporin; steroidal anti-inflammatory drugs, in particular dexamethasone, methylprednisolone; inhibitors of the angio-tensin-converting enzyme (ACE), in particular enalapril, captopril.

The administration of the compounds of the present invention can be carried out by oral, parenteral or transdermic way and they are generally administered simultaneously, successively or previously to the drug not containing the nitroderivative group which causes the gastrointestinal and/or renal and/or respiratory toxicity. The transdermic way is the preferred one and the compounds of the invention are administered under the form of patches or plasters. In particular conventional patches based on nitroglycerine are preferred, according to an embodiment of the present invention.

The dosages are the conventional ones already utilized for the DON-NO for the cardiovascular indications in human therapy. A commercial patch is generally utilized for one day or two days and then replaced. Slow release-patches could be

used for more days before being replaced. Sometimes also two patches a day, each for twelve hours, can be utilized. This procedure is generally preferred when a greater effectiveness is required.

Such dosages are preferred since they do not cause significant side effects as those typical of this class of drugs, for instance cephalgia, marked hypertension, etc.

The dosage ranges for the human therapy generally vary between 5-15 mg/24 h in 1-2 applications.

The compounds of the invention containing the  $-\text{ONO}_2$  functions or the  $-\text{NO}$  group producing the effects of the invention, as already said, must meet the test in vitro defined herein in detail.

In particular the test relates to the generation of nitric oxide from the NO donors of the present invention, among which, for instance, nitroglycerine, nicorandil, nitro-prussiate, etc., when they are put in the presence of endothelial cells (method a), or platelets (method b).

a) Endothelial cells

Cells of the human umbilical vein, spread on the plate, with density of  $10^3$  cells/plate were incubated with scalar concentrations of NO donor (1-100  $\mu\text{g/ml}$ ) for 5 minutes. The incubation medium (physiologic solvent, for instance Tyrode) was then analyzed to determine the capacity to generate NO, by means of:

- 1) the determination of nitric oxide by chemiluminescence;
- 2) the cGMP determination (cyclic GMP No. 2715 of the above mentioned Merck).

As regards the analysis by chemiluminescence, an amount equal to 100  $\mu$ l was injected in the reaction chamber of a chemiluminescence analyser containing glacial acetic acid and potassium iodide. The nitrites/nitrates present in the medium in these conditions are converted into NO which is then determined after its reaction with ozone, with consequent generation of light. As it usually occurs in the devices measuring chemiluminescence, the produced luminescence is directly proportional to the NO levels generated and can be measured by the suitable photomultiplier unit of a chemiluminescence analyser. The photomultiplier converts the incident light into electric voltage, which is then quantitatively recorded. On the basis of a calibration curve, prepared with scalar concentrations of nitrite, it was possible to determine quantitatively the generated NO concentration. For instance, from the incubation of 100  $\mu$ moles of nicorandil, an amount equal to about 10  $\mu$ moles of NO was generated.

As regards the cGMP determination, a portion of the incubation medium (equal to 100  $\mu$ l) was centrifuged

at 1000 revolutions for 20 seconds. The supernatant was discharged and the sediment taken again with iced phosphate buffer (pH 7.4). The cGMP levels produced were tested, by specific immuno-enzymatic reactants. From such experiments it resulted that, in these experimental conditions, the incubation with one of the various tested NO donors, caused a significant increase of cGMP with respect to the values obtained in absence of a NO donor. For instance, further to incubation with 100  $\mu$ moles of sodium nitroprussiate, an increase of about 20 times the value obtained with the incubation of only the vehicle without the NO donor was recorded.

b) Platelets

Washed human platelets, prepared analogously with what described by Radomski et al, (Br. J. Pharmacol. 92, 639-1987), were utilized. Aliquots of 0.4 ml were incubated with scalar concentrations of NO donors (1-100  $\mu$ g/ml) for 5 minutes. The incubation medium (f.i. Tyrode) was then analysed to determine the capacity of generating NO, by determination of nitric oxide by chemiluminescence and cGMP, with the modalities described in the previous paragraph, for the analyses carried out on the endothelial cells. As to the determination by chemiluminescence, also in this case, on the basis of a calibration curve, prepared with scalar concentrations

Of nitrite, it was possible to determine quantitatively the concentration of generated NO. For instance, after incubation of 100  $\mu$ moles of nicorandil, an amount equal to 35  $\mu$ moles of NO was generated.

As regards the cGMP determination, also in these experimental conditions, it resulted that the incubation with one of the various NO donors tested caused a significant increase of cGMP with respect to the values obtained in absence of a NO donor. For instance, after incubation with 100  $\mu$ moles of sodium nitroprussiate, an increase of about 30 times the value obtained with the incubation of only the vehicle without the NO donor, was recorded.

In conclusion, from said tests it results that all the NO donors according to the present invention, after incubation with endothelial cells or platelets for 5 minutes, are capable to generate NO, and to activate the cGMP synthesis in a concentration-dependent way, as determined by the utilized specific tests.

The following examples are given for illustrative purpose but are not limitative of the present invention.

EXAMPLESEXPERIMENTAL STUDIES ON COMBINATIONS BASED ON POTENTIALLY TOXIC DRUGS AND ON NO DONORS (INDICATED BY DON-NO)A) ANIMALS STUDIES

## 1) STUDY OF THE RENAL FUNCTIONALITY AFTER ADMINISTRATION OF ANTI-TUMORAL COMPOUNDS (CISPLATIN):

Sprague-Dawley male rats were daily treated with vehicle (physiologic saline solution, 0.9% sodium chloride, intraperitoneal (i.p.)) or cisplatin (i.p.) (5 mg/kg). Some animals received a daily dose of a NO donor, sodium nitroprussiate 0.2-1 mg/kg subcutaneous (s.c.). After five days the animals were sacrificed and the plasmatic urea and the plasmatic concentration of creatinine were determined. The data were analysed according to the bio-statistic methods commonly used.

As shown in Table 1, it resulted that the rats treated with cisplatin only showed meaningfully high levels of plasmatic urea and of creatinine, with respect to the control values (group receiving only the vehicle).

On the contrary, in animals, to which cisplatin and NO donor were administered, the biochemical parameters did not result meaningfully different from the control values.

## 2) STUDY OF THE RENAL FUNCTIONALITY AFTER ADMINISTRATION OF IMMUNO-DEPRESSIVE COMPOUNDS (CYCLOSPORIN):

Sprague-Dawley male rats were daily treated with vehicle (physiologic saline solution, 0.9% sodium chloride, i.p.) or intraperitoneal cyclosporin (5 mg/kg i.p.). Some animals received a daily dose of a NO donor, sodium nitroprussiate 0.2-1 mg/kg s.c. After eighteen days the animals were sacrificed and the plasmatic concentration of creatinine and the activity of N-acetyl-beta D-glycosaminidase (NAG) in the urines were measured. The data were analysed according to the bio-statistic methods commonly used.

As shown in Table 2, it resulted that the rats treated with cyclosporin only showed meaningfully high levels of blood creatinine and of above urine NAG with respect to the control values (group receiving only the vehicle).

On the contrary, in animals, to which cyclosporin and DON-NO donor were administered, the biochemical parameters did not result meaningfully different from the control values.

### 3) STUDY OF THE RENAL FUNCTIONALITY AFTER ADMINISTRATION OF ANTI-VIRAL COMPOUNDS (ACYCLOVIR):

Sprague-Dawley male rats were treated with vehicle (physiologic saline solution, 0.9% sodium chloride, i.p. a day) or intraperitoneal acyclovir (150 mg/kg i.p. a day). Some animals received a daily dose of a



DON-NO (nitroglycerine 1-10 mg/kg s.c. a day). After fifteen days the animals were sacrificed and the plasma concentration of creatinine was determined. The data were analysed according to the conventional biostatistic methods commonly used.

As shown in Table 1, it resulted that the rats treated with only acyclovir showed meaningfully high levels of blood creatinine with respect to the control values (group receiving only the vehicle).

On the contrary, in animals, to which acyclovir and DON-NO were administered, the biochemical parameters did not result meaningfully different from the control values (group receiving only the vehicle).

4) STUDY OF THE RENAL FUNCTIONALITY AND OF THE GASTROINTESTINAL TOLERABILITY IN ARTHRITIC RATS AFTER ADMINISTRATION OF NON-STEROIDAL ANTI-INFLAMMATORY COMPOUNDS (IBUPROFEN, NAPROXEN, INDOMETHACIN, DICLOFENAC) OR ANTI-THROMBOTICS (ASPIRIN):

Sprague-Dawley female rats were rendered arthritic, by an intracaudal injection of butyric *Micobacterium* inactivated by heat (0.6 ml suspended in 0.1 ml of mineral oil). After eighteen days, when the arthritic pathology was fully developed, the animals were daily treated with the vehicle (physiologic saline solution, 0.9% sodium chloride, i.p. a day) or NSAID [ibuprofen



(60 mg/kg i.p. a day); indomethacin (10 mg/kg/ i.p. a day); diclofenac (12 mg/kg i.p. a day; or naproxen (12 mg/kg i.p. a day)] or aspirin (250 mg/kg i.p. a day). Some animals received a daily dose of a DON-NO (sodium nitroprussiate 0.2-1 mg/kg s.c.; or nitroglycerin 1-10 mg/kg s.c. a day). After five days the animals were sacrificed and the plasmatic concentration of creatinine was determined. The data were analysed according to the conventional bio-statistic methods commonly used. As shown in Table 3, it resulted that the rats treated with only NSAID or aspirin showed meaningfully high levels of blood creatinine with respect to the control values (group receiving only the vehicle); such animals showed also a marked pathology affecting the gastrointestinal apparatus, having a severity ranging from the mucous erosion to ulcer involving the muscular layer, intestinal adhesences, abscesses, peritonitis. In the other groups, treated with the vehicle or combining DON-NO plus NSAID or aspirin, the pathology was either of much smaller entity or even absent. Moreover in the animals to which a NSAID or aspirin and a DON-NO were administered, the biochemical parameter did not result significantly different from the control values.

- 5) STUDY OF THE RENAL FUNCTIONALITY AND OF THE GASTROINTESTINAL TOLERABILITY IN HYPERTENSIVE RATS, AFTER ADMINISTRATION OF NON-STEROIDAL ANTI-INFLAMMATORY COMPOUNDS (DICLOFENAC) :

Sprague-Dawley male rats, spontaneously hypertensive (with systolic pressure variable between 180-220 mmHg) were daily treated with the vehicle (physiologic saline solution, 0.9 sodium chloride, i.p.) or NSAID[diclofenac(12 mg/kg i.p.)]. Some animals received a daily dose of an organic nitrate (nitroglycerin 1-10 mg/kg s.c. a day). After five days the animals were sacrificed and the plasmatic concentration of creatinine was determined. The data were analysed according to the conventional bio-statistic methods commonly used.

As shown in Table 4, it resulted that the rats treated with NSAID only showed meaningfully high levels of blood creatinine with respect to the control values (group receiving only the vehicle); such animals showed at the postmortem examination also a marked pathology affecting the gastrointestinal apparatus, of severity variable from the mucous erosion to ulcer involving the muscular layer, intestinal adhesences, abscesses, peritonitis. In the other groups, treated with the vehicle or combining DON-NO plus NSAID, the pathological picture affecting the gastrointestinal apparatus was

either of much smaller entity or even absent.

Moreover in the animals to which diclofenac and DON-NO were administered, the biochemical parameter did not result significantly different from the control values.

6) STUDY OF THE GASTROINTESTINAL TOXICITY AFTER ADMINISTRATION OF STEROIDAL ANTI-INFLAMMATORY COMPOUNDS (METHYLPREDNISOLONE):

Sprague-Dawley male rats were daily treated with the vehicle (physiologic saline solution, 0.9 sodium chloride, i.p.) or intraperitoneal methylprednisolone (5-10 mg/kg i.p.).

Some animals received a daily dose of a DON-NO (sodium nitroprussiate 0.2-1 mg/kg s.c.). After eighteen days the animals were sacrificed.

At the postmortem examination it resulted (Tab. 5) that such rats showed a marked pathology affecting the gastrointestinal apparatus, of severity variable from the mucous erosion to ulcer involving the muscular layer, intestinal adhesences, abscesses, peritonitis. In the other groups, treated with the vehicle only or with the combination nitrate plus steroid, the pathology was either of much smaller entity or even absent.

7) STUDY OF THE EFFECTS OF NITROXYBUTYLNAPROXEN (NO-NAPROXEN) ON CAPSAICIN INDUCED BRONCHOCONSTRICTION IN ENALAPRIL-TREATED GUINEA PIGS

Capsaicin-induced bronchoconstriction in guinea pigs is an animal model related to the ability of angiotensin-converting-enzyme inhibitors to provoke cough in patients (Subissi et al, J. Cardiovasc. Pharmacol. 20/1, 139-146, 1992).

NO-naproxen (2-(6-methoxy-2-naphthyl)propionate of 4-hydroxy-butyl) was synthesized according to Ex. 1, formula V) of International patent WO 95/09831.

Experimental conditions were as previously described by Del Soldato et al (J. Pharmacological Methods 5, 279, 1981). Female guinea pigs weighing 300-400 g were anesthetized through intraperitoneal injection of sodium 5,5 diethylbarbiturate (200 mg/kg) and kept under artificial respiration at constant positive pressure. Jugular right vein was cannulated for the administration of the compounds. Animals received intraduodenally enalapril (10 mg/kg), vehicle (carboxymethyl cellulose 2% by weight) and/or NO-naproxen (10 mg/kg). Forty-five minutes later, it was injected intravenously 0.1 ml capsaicin (1 µg/kg). Before and after capsaicin injection, tidal air changes were measured by means of modified Konzett apparatus connected to a polygraph amplifier.

Results were calculated as ratio of the responses obtained before and after the administration of each

treatment, expressed as % of the vehicle (control) response and shown in Table 7.

As shown in Table 7, NO-naproxen was able to reduce capsaicin-induced bronchoconstriction in enalapril treated guinea pigs. Enalapril increased capsaicin-induced bronchoconstrictive response, when administered alone.

B) STUDY ON PATIENTS

STUDY OF THE RENAL FUNCTIONALITY IN PATIENTS AFTER ADMINISTRATION OF ANTI-TUMORAL DRUGS (CISPLATIN).

In some patients, separately observed, and in uncontrolled studies was evaluated the acute effect of some drugs such as cisplatin, alone or in the presence of a nitroglycerin patch.

The mono-administration of intraperitoneal cisplatin (90 mg per m<sup>2</sup>) to patients, which needed an antitumoral therapy, caused a significant increase of blood creatinine in the first 24 hours, with respect to the initial values.

As it results from Table 6, when the patients were submitted to daily co-treatment with the nitroglycerin patch approximately releasing 15 mg/24 hours of nitroglycerin when the patch came into contact with the skin, such increase was much more limited and however significantly not different from the initial values.

The data were analysed according to the conventional biostatistic methods commonly used.

**TABLE 1**

STUDY OF THE RENAL FUNCTIONALITY IN RATS, AFTER THE REPEATED TREATMENT WITH CISPLATIN OR ACYCLOVIR, IN THE PRESENCE OR NOT OF NO DONOR. THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE CONTROL VALUE (GROUP TREATED WITH ONLY THE VEHICLE).

TREATMENT	BLOOD UREA	BLOOD CREATININE
VEHICLE	100	100
CISPLATIN	683*	245*
CISPLATIN+DON-NO	142	120
ACYCLOVIR	-	208*
ACYCLOVIR+DON-NO	-	104

\*P< 0.05 with respect to the control values.

**TABLE 2**

STUDY OF THE RENAL FUNCTIONALITY IN ARTHRITIC RATS AFTER THE REPEATED TREATMENT WITH CYCLOSPORIN IN THE PRESENCE OR NOT OF A DON-NO. THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE CONTROL VALUE (GROUP TREATED WITH ONLY THE VEHICLE)

TREATMENT	NAG	BLOOD CREATININE
VEHICLE	100	100
CYCLOSPORIN	220*	187*
CYCLOSPORIN+DON-NO	85	110

\*P<0.005 with respect to the control values

TABLE 3

STUDY OF THE RENAL FUNCTIONALITY IN ARTHRITIC RATS AFTER THE REPEATED TREATMENT WITH SOME ANTI-INFLAMMATORY COMPOUNDS, IN THE PRESENCE OR NOT OF DON-NO. THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE CONTROL VALUES (GROUP TREATED WITH ONLY THE VEHICLE)

TREATMENT	BLOOD CREATININE
VEHICLE	100
IBUPROFEN	292*
IBUPROFEN+ SODIUM NITROPRUSSATE (0.5 mg/kg s.c.)	123
IBUPROFEN+ NITROGLYCERIN (3 mg/kg s.c.)	142
INDOMETHACIN	355*
INDOMETHACIN+ SODIUM NITROPRUSSATE (0.5 mg/kg s.c.)	138
INDOMETHACIN+ NITROGLYCERIN (3 mg/kg s.c.)	130
DICLOFENAC	371*
DICLOFENAC+ NITROGLYCERIN (3 mg/kg s.c.)	122
NAPROXEN	323*
NAPROXEN+ NITROGLYCERIN (3 mg/kg s.c.)	164
ASPIRIN	280*
ASPIRIN+ NITROGLYCERIN (3 mg/kg s.c.)	112

\*P< 0.05 with respect to the control values



**TABLE 4**

STUDY OF THE RENAL FUNCTIONALITY IN HYPERTENSIVE RATS, AFTER THE REPEATED TREATMENT WITH DICLOFENAC, IN THE PRESENCE OR NOT OF DON-NO. THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE CONTROL VALUES (GROUP TREATED WITH ONLY THE VEHICLE)

TREATMENT	BLOOD CREATININE
VEHICLE	100
DICLOFENAC	287*
DICLOFENAC+ NITROGLYCERIN (3 mg/kg s.c.)	148

\*P< 0.05 with respect to the control values

**TABLE 5**

STUDY OF THE GASTROINTESTINAL TOLERABILITY IN RATS, AFTER THE REPEATED TREATMENT WITH METHYLPREDNISOLONE, IN THE PRESENCE OR NOT OF A DON-NO. THE SEVERITY DEGREE OF THE GASTROINTESTINAL PATHOLOGY WAS EVALUATED ACCORDING TO THE USUAL METHODS AND EXPRESSED IN ARBITRARY VALUES.

THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE CONTROL VALUES (GROUP TREATED WITH ONLY THE VEHICLE)

TREATMENT	GASTROINTESTINAL HARM
VEHICLE	100
PREDNISOLONE	683*
PREDNISOLONE+SODIUM NITROPRUSSATE (0.5 mg/kg s.c.)	142

\*P< 0.05 with respect to the control values



TABLE 6

STUDY OF THE RENAL FUNCTIONALITY IN ONCOLOGIC PATIENTS, AFTER THE TREATMENT WITH CISPLATIN, IN THE PRESENCE OR NOT OF A DON-NO. THE DATA ARE EXPRESSED AS PERCENT VARIATION WITH RESPECT TO THE INITIAL VALUES.

TREATMENT	BLOOD CREATININE VALUES	
	INITIAL	FINAL
CISPLATIN	100	183*
CISPLATIN+ NITROGLYCERIN PATCH	100	109

\*P< 0.05 with respect to the control values.

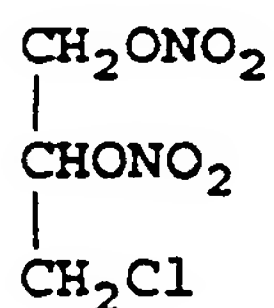
TABLE 7

EFFECTS ON NITROXYBUTYLNAPROXEN (NO-NAPROXEN) ON CAPSAICIN INDUCED BRONCHOCONSTRICTION IN ENALAPRIL-TREATED GUINEA PIGS

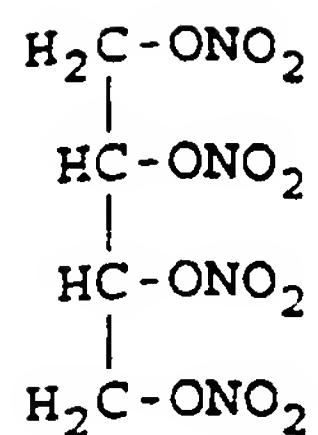
TREATMENT	BRONCHOCONSTRICTION (%)
VEHICLE	100
ENALAPRIL	290
ENALAPRIL + NO-NAPROXEN	20

## C L A I M S

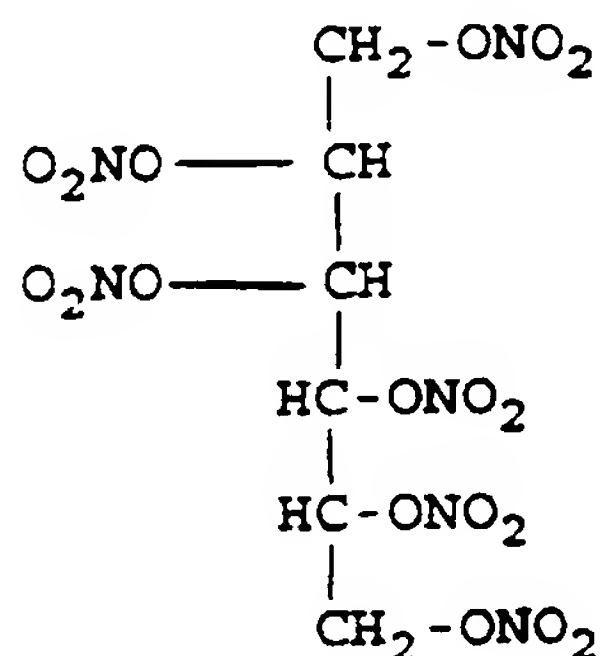
1. Use of organic compounds containing the  $-\text{ONO}_2$  function, or inorganic compounds containing the  $-\text{NO}$  group or compositions comprising said compounds to reduce the toxicity caused by drugs not containing nitroderivative groups to the gastrointestinal and/or renal and/or respiratory apparatus, said compounds being characterized in that they are nitric oxide NO donors, that is when they are put into contact in vitro with cells of the basal endothelium or platelets, and after incubation of 5 minutes at the temperature of  $37^\circ\text{C}$  are capable of releasing NO and activating the cGMP (Guanosine cyclic 3',5'-(hydrogen phosphate)) synthesis.
2. Use of compounds according to claim 1, wherein the organic compounds containing the  $-\text{ONO}_2$  function are selected among:  
clonitrate (3-chloro-1,2-propanediol dinitrate) having the formula  $\text{C}_3\text{H}_5\text{ClN}_2\text{O}_6$  and formula of structure



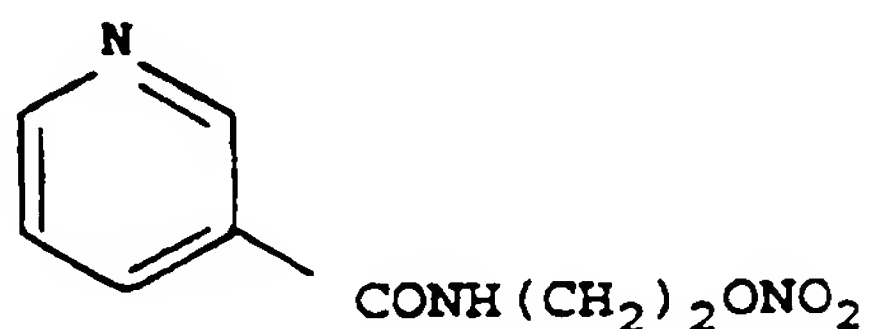
erythrityltetranitrate (1,2,3,4 butanetetroltetranitrate) having the formula  $\text{C}_4\text{H}_6\text{N}_4\text{O}_{12}$  and formula of structure



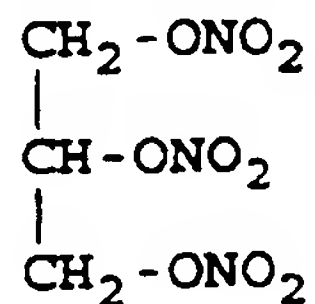
mannitol hexanitrate having the formula  $\text{C}_6\text{H}_8\text{N}_6\text{O}_{18}$  and formula of structure



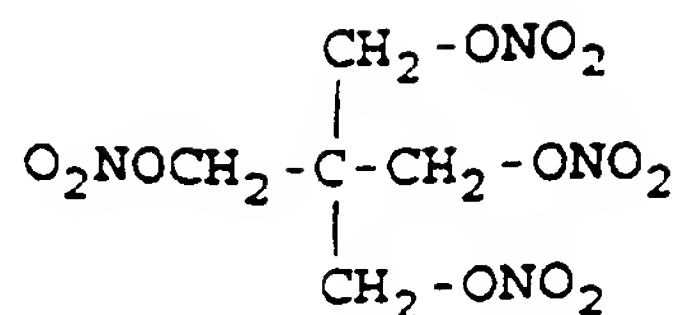
nicorandil (N-[2-(nitrooxy)ethyl]-3-pyridine-carboxamide) having the formula  $\text{C}_8\text{H}_9\text{N}_3\text{O}_4$  and formula of structure



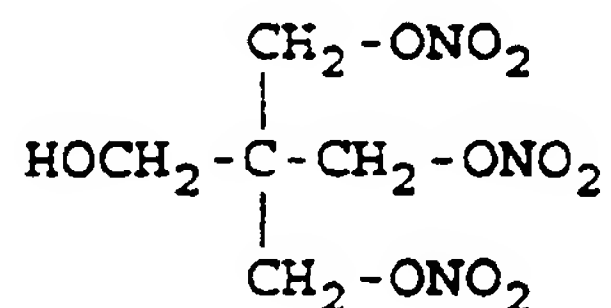
nitroglycerin (1,2,3 propanetriol trinitrate) having the formula  $\text{C}_3\text{H}_5\text{N}_3\text{O}_9$  and formula of structure



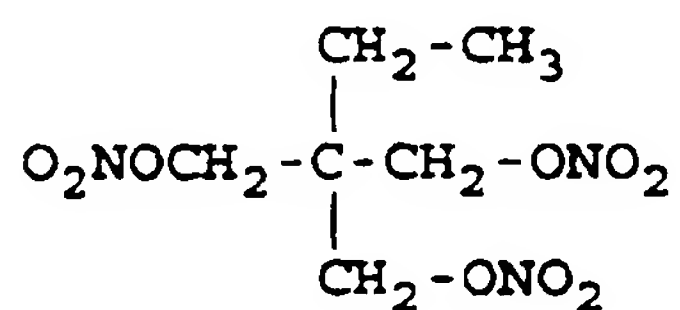
pentaerythritoltetranitrate (2,2-bis [(nitrooxy)-methyl]-1,3-propanedioldinitrate) having the formula  $\text{C}_5\text{H}_8\text{N}_4\text{O}_{12}$  and formula of structure



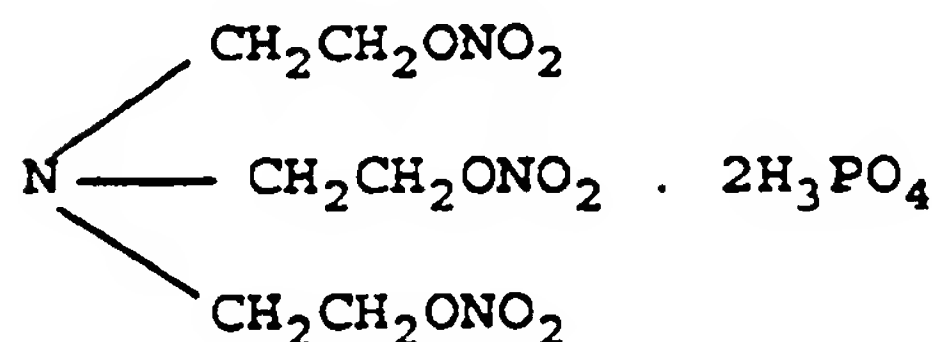
pentrinitrol (2,2-bis[(nitrooxy)methyl]-1,3-propanediolmononitrate) having the formula  $\text{C}_5\text{H}_9\text{N}_3\text{O}_{10}$  and formula of structure



propatylnitrate (2-ethyl-2-[(nitrooxy)methyl]-1,3-propanedioldinitrate) having the formula  $\text{C}_6\text{H}_{11}\text{N}_3\text{O}_9$  and formula of structure



trolnitratephosphate (2,2',2"-nitryltrisethanoltrinitrate phosphate) (salt 1:2) having the formula  $\text{C}_6\text{H}_{18}\text{N}_4\text{O}_{17}\text{P}_2$  and formula of structure



3. Use of compounds according to claim 1 wherein the inorganic compounds containing the -NO group are selected from nitroprussiates.
4. Use of compounds according to claim 3, wherein the inorganic compounds containing the -NO group are sodium

nitroprussiate (pentakis(cyano-C)nitrosylferrate(2-)disodium) having formula  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$  .

5. Use of compounds according to claims 1-4 wherein the drugs not containing nitroderivative groups, causing renal and/or gastrointestinal and/or respiratory toxicity belong to the following therapeutic classes:  
anti-tumoral, immunodepressive, anti-viral drugs,  
non-steroidal anti-inflammatory drugs, anti-thrombotic drugs, steroidal anti-inflammatory drugs, antibiotics, inhibitors of the angiotensin-converting enzyme.
6. Use of compounds according to claim 5 wherein the drugs are chosen from:  
anti-tumoral drugs chosen between cisplatin, 5 fluoro-uracil;  
immunodepressive drugs chosen from cyclosporin;  
anti-viral drugs chosen from acyclovir;  
non-steroidal anti-inflammatory drugs chosen among ibuprofen, indomethacin, diclofenac, ketorolac, naproxen, ketoprofen, mefenamic acid, flunixin, flufenamic acid, niflumic acid;  
anti-thrombotic drugs chosen from aspirin;  
steroidal anti-inflammatory drugs chosen among cortisone, dexamethasone, methylprednisolone;  
antibiotics chosen between ciprofloxacin, gentamicine;  
inhibitors of the angiotensin-converting enzyme chosen

- between captopril, enalapril;  
beta-adrenergic antagonists, chosen among atenolol,  
metoprolol, timolol, propanol.
7. Use of compounds according to claims 5 and 6, wherein the drugs are chosen from:  
the antitumoral is cisplatin, the immunodepressive is cyclosporin, the steroidal anti-inflammatory drugs are dexametasone, methylprednisolone.
  8. Use of compounds according to claims 1-7, wherein the administration of the NO donor compounds is carried out by oral, parenteral or transdermic way.
  9. Use of compounds according to claim 8 wherein the administration of NO donor compounds is carried out simultaneously, successively or previously to the drug causing the gastrointestinal and/or renal and/or respiratory toxicity.
  10. Use of compounds according to claim 9 wherein the administration of NO donor compounds is carried out by transdermic way under the form of patches or plasters.
  11. Use of compounds according to claim 10 wherein the patches are based on nitroglycerine.
  12. Use of compounds according to claims 1-11, wherein the dosages are those used for the nitroderivative compounds for cardiovascular applications in human therapy.

## INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCI/EP 97/00873

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J GASTROENTEROL HEPATOL, 1994, 9 SUPPL 1 PS40-4, AUSTRALIA, XP000675240 WALLACE JL ET AL: "Nitric oxide-releasing non-steroidal anti-inflammatory drugs: a novel approach for reducing gastrointestinal toxicity." see page S41, column 1, paragraph 2 - column 2, paragraph 2 ---	1,3-5,8, 9
A	WO 96 00073 A (MASSACHUSETTS EYE AND EAR INFI ;DREYER EVAN B (US)) 4 January 1996 see abstract see page 4, line 14 - line 17 -----	1-12



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

6 June 1997

Date of mailing of the international search report

20.06.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Leherte, C

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC/EP 97/00873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
---	---------------------	----------------------------	---------------------

WO 9600073 A	04-01-96	US 5597809 A	28-01-97
		AU 2819695 A	19-01-96
		CA 2192209 A	04-01-96

-----